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A61K 31/70

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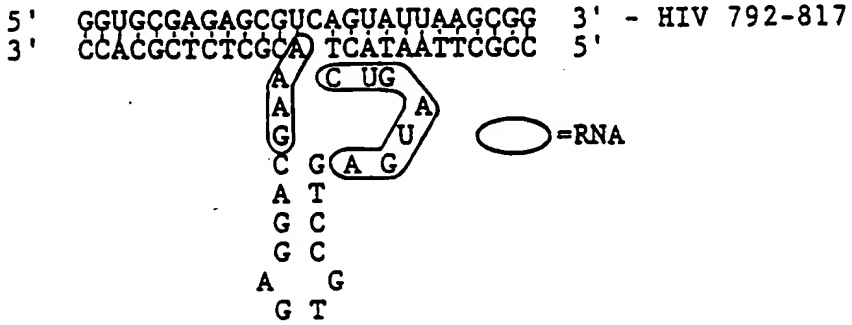
(81) Designated States: AU, CA, DE*, FR (European patent), GB, IT (European patent), JP, US.

Published
With international search report.

(54) Title: CHIMERIC DNA-RNA CATALYTIC SEQUENCES

DRDRD-1



(57) Abstract

This invention provides chimeric DNA/RNA catalytic molecules useful to cleave RNA sequences. The invention specifically provides two different chimeric DNA-RNA-DNA-RNA-DNA catalytic molecules which are targeted to cleave HIV-1 RNA sequences. These chimeric molecules include DNA sequences which flank a catalytic RNA center. Interaction with the HIV-1 substrate RNAs is achieved by Watson-Crick base pairing of the DNA flanking sequences with HIV-1 RNA. The catalytic ribonucleotide center cleaves the phosphodiester bond of the substrate HIV-1 RNA at the expected location.

* See back of page

Summary of the Invention

This invention provides chimeric DNA/RNA catalytic molecules useful to cleave RNA sequences. The invention specifically provides two different chimeric DNA-RNA-DNA-RNA-DNA catalytic molecules which are targeted to cleave HIV-1 RNA sequences. These chimeric molecules include DNA sequences which flank a catalytic RNA center. Interaction with the HIV-1 substrate RNAs is achieved by Watson-Crick base pairing of the DNA flanking sequences with HIV-1 RNA. The catalytic ribonucleotide center cleaves the phosphodiester bond of the substrate HIV-1 RNA at the expected location.

General Description of the Invention

In general the catalytic molecules of the invention function as hammerhead or hairpin ribozymes. The preferred molecular construct consists of two known RNA catalytic sequences each flanked by a DNA sequence at the respective 3′ and 5′ termini and coupled by a DNA sequence at the corrseponding 5′ and 3′ termini. These molecules may accordingly be represented by the formulae I and II::

I. 3′ X - AAAG - Y - AGUAGUC - Z 5′

or

II. 3′ X - CAAAG - Y - AGUAGUC - Z 5′

in which X, Y and Z are DNA sequences and AAAG, CAAAG and AGUAGUC are catalytic RNA sequences.

The flanking X and Z components may be any DNA sequences that allow base pairing with the substrate RNA at appropriate positions adjacent to the substrate cleavage site. These flanking sequences may be phosphodiester, phosphorothioate, methyl phosphonate, methyl phosphate or similar moieties.

Y may be any DNA sequence that base pairs inter se in the manner required for catalytic cleavage of

the substrate by the RNA sequences preferably as shown in base paired form in Formula III:

III. 5′ C-G 3′
A-T
G-C
G-C
A G
G T

The catalytic molecules of this invention can be synthesized in known manner by commercially available DNA synthesizers such as those produced by Applied Biosystems or Milligen. See, e.g., Perreault, et al, supra.

The X and Z sequences may be substituted at the respective 3′ and 5′ ends with ligands to facilitate cell entry, targeting within the cell and ultimate stability of the catalysts. Such ligands include by way of example but not of limitation: other nuclotides, proteins, carbohydrates, lipids, steroid hormones and cholesterol.

The catalytic molecules of the invention are administered by known and available delivery agents or systems, including, but not limited to, liposomes, defective viral particles, viral capids, and standard DNA/RNA transfective procedures.

Description of the Figures

Figure 1 illustrates one catalytic molecule of the invention base paired to an HIV-1 sequence. The RNA portion of the molecule is encircled.

Figure 2 illustrates a second catalytic molecule of the invention base paired to another HIV-1 sequence. The RNA portion of the molecule is encircled.

Figure 3A depicts a ribonuclease A digestion of the catalytic molecule of Figure 1 as compared with an equivalent all DNA molecule. The conditions were

10 units of commercial (Sigma) pancreatic ribonuclease in 2XSSC buffer added t the oligonucle tides which were in 10 microliters of 50 mM Tric-HCl buffer (pH 8.0). The RNAse was incubated with the sample for 10 minutes before the ^{32}-P end labelled DRDRD or DNA molecules were electrophoresed in a 15% polyacrylamide gel containing 8M urea. The gel was autoradiographed for 10 minutes to get the exposure depicted.

Figure 3B depicts a cleavage reaction involving the catalytic molecule of Figure 1 under conditions described in Chang, et al., Clinical Biotechnology, 2:23-31 (1990).

EXAMPLE I

The catalytic molecule of Figure 1 was synthesized in known manner utilizing an automated oligonucleotide synthesizer manufactured by Applied Biosystems, Inc.

The result of ribonuclease A digestion of the catalytic molecule is shown by Figure 3A.

The catalytic molecule produced, as described, was used to cleave each of a 610 nuleotide long (S-610) and a 170 nucleotide long HIV-1 gag transcript. In brief, the buffer was 50 mM Tris-HCl, pH 7.5, 1mM EDTA, 10mM $MgCl_2$ at approximately 1 pmole of target, 3 pmole of ribozyme or DNA. The reactions were carried out at 37°C. for 12 hours. The substrate was either a 610 nucleotide long HIV-1 gag containing transcript (S-610) or a 172 nucleotide long HIV-1 gag containing transcript (S-172). The 5′ cleavage product is indicated for both.

In Figure 3B the 5′ cleavage product is shown for both transcripts. The 3′ cleavage product for the 610 target is not visible due to poor reproduction of

the autoradiograph, but is indicated in its position by a 3′ P notation. As a negative control, an all DNA oligonucleotide (D) of the same sequence as the DRDRD molecule was incubated with the same substrates under the same conditions with the result that no cleavage was obtained.

Specific cleavage of an HIV-1 5′ LTR splice site with a similar catalytic molecule has also been obtained.

CLAIMS

1. A catalytic m lecule capable of cleaving an HIV-1 RNA sequence at a known rib zyme cleavage site said molecule having the formula

3′ X - AAAG - Y - AGUAAGUC - Z 5′

or

3′ X - CAAAG - Y - AGUAAGUC - Z 5′

in which X and Z are DNA sequences that base pair with an RNA substrate at positions juxtaposed to said known cleavage site,

AAAG, CAAAG and AGUAGUC are RNA sequences,

Y is a DNA sequence that base pairs inter se in a manner required to permit said RNA sequences to cleave said substrate at said cleavage site.

2. The catalytic molecule shown by Figure 1.

3. The catalytic molecule shown by Figure 2.

4. A catalytic molecule, as defined by Claim 1, in which said RNA sequence is an HIV-1 sequence.

5. A catalytic molecule, as defined by Claim 4, in which said HIV-1 sequence is the HIV-1 sequence shown by Figure 1.

6. A catalytic molecule, as defined by Claim 4, in which the HIV-1 sequence is the HIV-1 sequence shown by Figure 2.

7. A catalytic molecule capable of cleaving an RNA sequence, said molecule having catalytic RNA moieties linked to first and second DNA moieties which base pair with the substrate RNA sequences flanking the cleavage site and interconnected by a third DNA sequence which base pairs inter se to facilitate said cleavage.

FIG. 1 DRDRD-1

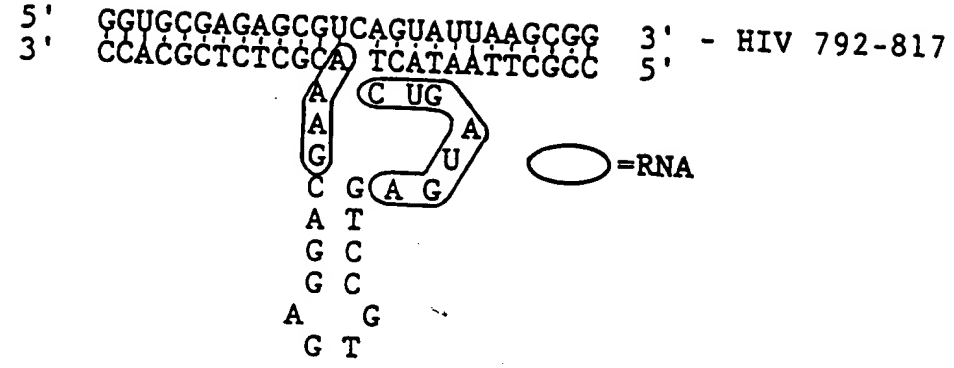


FIG. 2 DRDRD #2

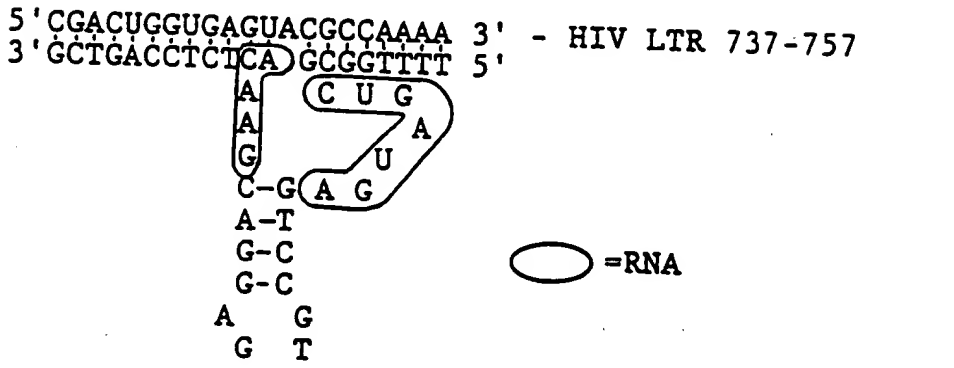


FIG. 3A

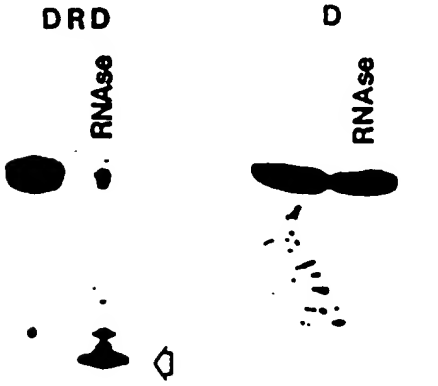
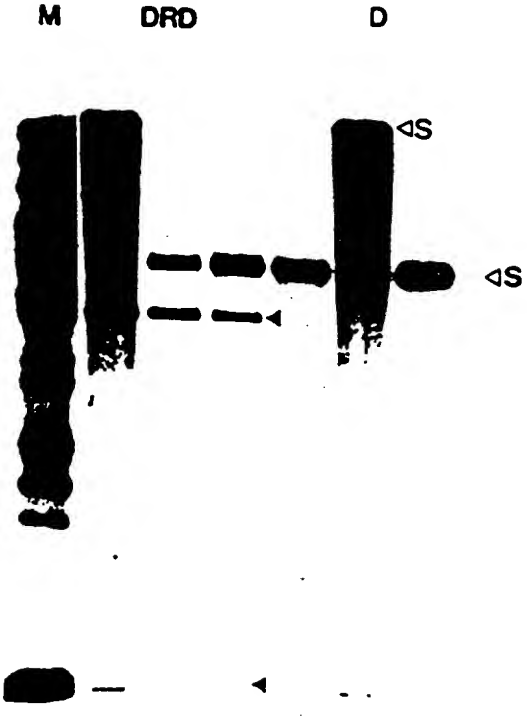


FIG. 3B



INTERNATIONAL SEARCH REPORT

International Application No PCT/US90/03102

I. CLASSIFICATION OF SUBJECT MATTER (If several classification symbols apply, indicate all) [3]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC(5): A16K 37/62; C07H 17/00, 15/12; A61K 31/70

U.S.Cl.: 424/94.6; 536/23, 29; 514/44

II. FIELDS SEARCHED

Minimum Documentation Searched [4]

Classification System	Classification Symbols
U.S.Cl.	424/94.6; 536/23, 29; 514/44

Documentation Searched other than Minimum Documentation to the Extent that such Documents are included in the Fields Searched [5]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [14]

Category [6]	Citation of Document, [16] with indication, where appropriate, of the relevant passages [17]	Relevant to Claim No. [18]
A,P	Chemical Abstract, Volume 112, No. 7, issued 12 February 1990 (Columbus, Ohio, U.S.A.) W. Gerlach, et al, "Synthetic Ribozymes for in ViVo Inactivation of Prokaryotic or Eukaryotic RNA Transcripts", See pages 336-337, column 2, See the abstract No. 51284j, Eur. Pat. Appl. EP 321,201 21 June 1989.	1 - 7
A,P	Chemical Abstract, Volume 112, No. 19, issued 07 May 1990 (Columbus, Ohio, U.S.A.) N. Sarver, et al, "Ribozymes as Potential Anti-HIV-1 Therapeutic Agents", See page 420, column 2, See the abstract No. 17548q, Science, 1990, 247 (4947), 1222-5(Eng).	1 - 7
A,P	Chemical Abstract, Volume 112, No. 7, issued 12 February 1990 (Columbus, Ohio, U.S.A.), M. Cotten, et al, "Ribozyme Mediated Destruction of RNA in ViVo", See page 501, column 1, See the abstract No. 52942j, EMBO J, 1990, 8(12), 3861-6 (Eng)	1 - 7

* Special categories of cited documents: [15]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search [3]	Date of Mailing of this International Search Report [3]
07 AUGUST 1990	05 DEC 1990
International Searching Authority [1]	**Signature of Authorized Officer [19]**
ISA/US	John W. Rollins

Form PCT/ISA/210 (second sheet) (May 1986)

cdb:8/11/90

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, ** with indication, where appropriate, of the relevant passages **	Relevant to Claim No **
A,P	Nature, volume 344, issued 05 April 1990, J. Peneault, et al., Mixed Deoxyribo - and Ribooligonucleotides with Catalytic activity see pages 565-567.	1-7
A,P	Proceeding of the National Academy of Sciences, Volume 86, no. 23, issued December 1989 (U.S.A.) F.H. Cameron, et al., 'Specific Gene Suppression by Engineered Ribozymes in Monkey Cells', see pages 9139 - 9143.	

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

A	Chemical Abstracts, Volume 110, No. 21, issued 22 May 1989, (Columbus, Ohio, U.S.A.) T. R. Cech et al., "RNA Ribozyme Polymerases, Dephosphorylases, Restriction Endoribonucleases and Methods for Their Manufacture", See page 226, column 2, See the abstract No. 187321K, PCT Int. Appl. WO8804,300 16 June 1988.	1 - 7

V. ☐ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE [1]

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. ☐ Claim numbers . because they relate to subject matter [1] not required to be searched by this Authority, namely:

2. ☐ Claim numbers . because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out [1], specifically:

3. ☐ Claim numbers_____, because they are dependent claims not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. ☐ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING [2]

This International Searching Authority found multiple inventions in this international application as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.

2. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

3. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

4. ☐ As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

☐ The additional search fees were accompanied by applicant's protest.

☐ No protest accompanied the payment of additional search fees.